

Nanotechnologies for biomedical science and translational medicine

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In 2000 the United States launched the National Nanotechnology Initiative and, along with it, a well-defined set of goals for nanomedicine. This Perspective looks back at the progress made toward those goals, within the context of the changing landscape in biomedicine that has occurred over the past 15 years, and considers advances that are likely to occur during the next decade. In particular, nanotechnologies for health-related genomics and single-cell biology, inorganic and organic nanoparticles for biomedicine, and wearable nanotechnologies for wellness monitoring are briefly covered.

nanotechnology | biotechnology | nanomedicine

When rowing a boat across a big lake, you can paddle for a long time, and the far shore will continue to appear almost as far away as when you started. However, when you look back to your starting point, the amount of distance traveled can be startling. In 2000, Neal Lane, then President Clinton's Science Advisor, presented the first Implementation Plan for the US National Nanotechnology Initiative (NNI) (1). That document laid out some clear goals for nanomedicine:

- i) Rapid, more efficient genome sequencing enabling a revolution in diagnostics and therapeutics
- ii) Effective and less expensive health care using remote and in vivo devices
- iii) New formulations and routes for drug delivery that enormously broaden their therapeutic potential by targeting the delivery of new types of medicine to previously inaccessible sites in the body
- iv) More durable rejection-resistant artificial tissues and organs
- v) Devices that enable vision and hearing aids
- vi) Sensor systems that detect emerging disease in the body, which ultimately will shift the focus of patient care from disease treatment to early detection and prevention.

The goals of the 2000 NNI have largely stood the test of time and even capture some of today's grand challenges in the health sciences. I'll look back at selected items from this list and provide brief descriptions of how far we have come. For most of these goals, nanotechnologies have played supporting, albeit gradually increasing, roles. For example, any discussion of diagnostics, early disease detection, and disease prevention

would likely lead with the amazing progress in the "-omics" fields and the emerging efforts to define "wellness" quantitatively that ultimately will provide the baseline against which disease is measured (2). However, a little digging reveals that nanotech is playing increasingly important roles in the emerging generation of omics tools. For other goals, such as those associated with new formulations and routes for drug delivery, nanotech has played a major role, even if widespread clinical applications are still on the horizon. Just like the rower's view from the lake, the various end goals of the NNI are still a ways off, but the distance covered is impressive.

Although the goals of the 2000 NNI still appear prescient, there have been major breakthroughs in medicine and biotechnology that weren't anticipated 15 y ago. For example, single-cell biology was not really a field in 2000, but now it is one of the most rapidly evolving assay biotechnologies, with potentially disruptive implications in terms of how we think about biological systems and how we understand human disease and health states. As a second example, until a couple of years ago the idea of harnessing a patient's immune system as a powerful anticancer drug was more or less a backwater of cancer research. Immune checkpoint inhibitors, which now are dominating new cancer clinical trials, with several recent Food and Drug Administration approvals, were only being identified in 2000 (3). However, cancer immunotherapy (4) in the forms of both checkpoint inhibitors and cell-based therapies is increasingly altering the dialogue of "cancer treatments" to "cancer cures." These two breakthrough areas relate somewhat to the Year 2000 NNI goals 1 and 3, respectively, although the supporting roles that

nanotech is playing in the development of these new fields are quite distinct from those anticipated 15 y ago.

This paper is a perspective rather than a proper review, so many topics are necessarily not covered. Of the six original NNI goals, I don't discuss numbers 4 and 5, and progress toward a couple of the others is mentioned only briefly. Even for those topics I do discuss, I am forced to neglect a great deal of great science, and for that I apologize in advance! In the following pages I stress both the fundamental scientific advances that are enabling the current state of the art of nanomedicine and the conceptual advances and biomedical needs that are driving the field. In the "bottom-up" spirit of nanotechnology, I begin with discussions of nanotech contributions to genomics and other -omics measurements, because those measurements are increasingly providing a foundation for modern medicine. Finally I briefly peer into the bright future of nanomedicine.

Genomics and Nanotechnology

Goal no. 1 of the 2000 NNI forecasts an important role for nanotech in enabling inexpensive sequencing. The rapid advancement in next-generation genome sequencing (NGS) technologies has been a triumph of modern biotechnology, so that the \$1000 genome is now a reality (5). In fact, the NGS tools have advanced to the point that the technology is effectively a given, and the

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intellectual effort within a genomics study now is centered on the scientific design and interpretation of population-based studies (6). However, NGS has major deficiencies. First, most NGS methods are limited to short reads, although alternative technologies are emerging that permit longer reads (7). Analyzing an NGS dataset is, by analogy, similar to reading a book in which short sentence fragments are placed in random order relative to how the text actually was written. To make it understandable, the book must be reassembled correctly from those short fragments. Second, NGS requires amplification. This requirement, combined with short reads, means that the identification of many natural or disease-associated molecular lesions, such as repeat-rich regions, gene amplifications and deletions, or chromosomal translocations, can be challenging. Finally, NGS requires expensive reagents. Thus, new sequencing or mapping technologies are emerging based on long reads of single DNA molecules, without the need for amplification or even reagents (8). These approaches are enabled by nanotechnologies (Fig. 1) with specific design parameters that draw from many years of polymer-based physics, biophysics, and physical chemistry.

Nanochannel-based single-molecule genome mapping (Fig. 1A) (9) is used to help assemble genomes and fill in information gaps that are not captured by NGS. The origins of the technique were methods in which single DNA molecules, 10^2 – 10^4 kb long, were stretched out on a microscope slide and cleaved using site-specific enzymes. In this way a 400-kb DNA molecule might be cut into 20–40 components (10). The physical length of the resulting segments, averaged over many DNA molecules similarly analyzed, provided a crude genome map (11). For nanochannels, the concept illustrated in Fig. 1A relies on fluorophores that are introduced by using nicking endonucleases that cut the dsDNA at specific restriction sites. These locations provide a map that includes deletions, amplifications, and translocations and provides guides for NGS genome assembly. Of course, many physical chemistry issues, such as the interplay of the persistence length of the DNA molecules, the ionic strength of the solvent, the conditions for uncoiling the DNA, and the dimensions of the nanochannel, have all provided a scientific foundation for this nanotechnology (12, 13).

Long, accurate sequencing reads of unamplified DNA would negate the need for

mapping. Nanopore-based sequencing (14) is such a method, although it has been technically challenging to develop and only very recently has permitted some sequence determination of an actual genome (15, 16). The basic idea dates back some 20 y and draws from fundamental work in surface science, molecular biology, nanofabrication, and electrochemistry (17). In one manifestation, two aqueous electrolyte solutions are connected through a single protein nanopore, such as such as *Mycobacterium smegmatis* porin A (MspA). A bias is applied, and current is measured as DNA is drawn through a bilayer-entrained pore protein at a rate that is controlled through the use of molecular motors such as Φ 29 DNA polymerase (DNAP) (18). A variant developed by Pacific Biosciences (19) is shown in Fig. 1B. Four distinct fluorophores are used to read out the action of the polymerase, which is isolated within a nanofabricated waveguide. The polymerase, which also is used for whole-genome amplification, permits long, relatively unbiased reads. Each polymerase permits a read rate of about four to five bases per minute, with thousands of single polymerases in nanowaveguides used in parallel. A third variant, which was released by Oxford Nanopore Technologies as the MinION nanopore sequencing product, is about the size of a USB memory stick. Early literature reports on the MinION imply that it may not yet be ready for wholesale sequencing (20), but it does have some compelling applications (21).

Although genomics has led the -omics revolution, nanotechnologies (and microfluidics) are playing increasingly important roles in reducing -omics technologies to the level of a single cell, and I turn to this area next.

Single-Cell -Omics in Biology and Biomedicine

Single-cell biology holds the promise of unraveling the heterogeneity that often confounds the interpretation of biological or biomedical measurements. For example, single-cell analysis is increasingly applied toward understanding immune responses triggered during various cancer immunotherapies (22–25) or for unraveling the functional behaviors that emerge from heterogeneous healthy or diseased tissues (26, 27). Aside from flow- and mass-cytometry methods (28, 29), virtually every single-cell -omics platform involves some level of microfluidics and/or nanotech. Specifically, a single cell has only a certain number of copies of any given analyte. By isolating a single cell within a nanoliter or subnanoliter volume, those copy numbers can correspond to detectable concentrations, and the concentrations of contaminants are minimized.

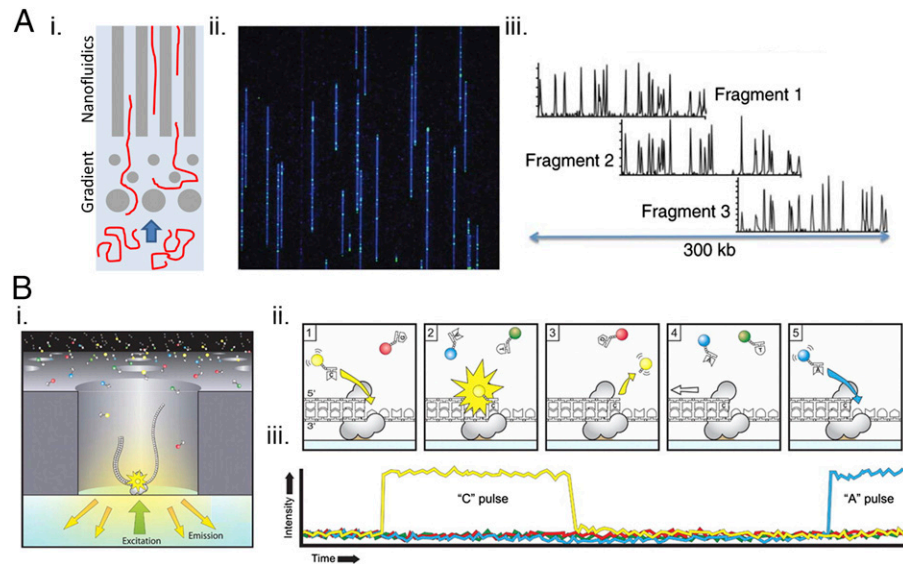


Fig. 1. Nanotechnologies for genome mapping and genome sequencing. (A) Nanochannel-based genome mapping. (i) The microchip is designed to untangle the DNA and guide long fragments into nanochannels with diameters <100 nm. (ii) The DNA fragments are fluorescently labeled at specific sites to provide a spatial map of those sites. (iii) Different fragments are lined up according to the spatially resolved fluorescent signatures to provide the genome map, shown here for a 300-kb segment. Adapted from ref. 9, with permission from Macmillan Publishers Ltd: *Nature Biotechnology*, copyright 2012. (B) The Pacific Biosciences nanopore-based genome-sequencing platform, in which single-molecule, real-time sequencing data are obtained from a DNA polymerase that is isolated within a 100-nm-diameter pore in an aluminum film. (i) The pore serves as a zero mode waveguide for optical analysis of uninterrupted template-directed DNA synthesis using four distinguishable fluorescently labeled dNTPs. (ii) The temporal order of enzymatic incorporation of the dNTPs into a growing DNA strand is illustrated. At each step a fluorescent dNTP is incorporated, generating a fluorescent signal that is collected efficiently within the nanowaveguide. The fluorophore then is cleaved and diffuses out of the waveguide. (iii) An optical readout for a single channel is illustrated. Reproduced from ref. 19, with permission from AAAS.

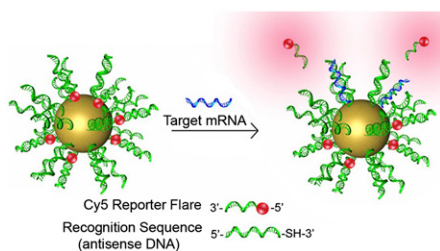


Fig. 3. The gold NP-based nanoflare construct is used for detecting specific mRNAs in live cells. The gold NPs are coated with a dense layer of DNA, which promotes cell penetration. The DNA shown comprises a fluorescent reporter (the Cy5 flare), which is nonfluorescent when bound to the Au NP. This nanoflare is hybridized with an antisense DNA. When the nanoflare encounters the target mRNA, the flare is released, thus activating fluorescence within the cell and permitting live-cell sorting based on the expression of a specific gene. Adapted from ref. 75.

more challenging. Each of the elements of this properties matrix [which includes the Stokes radius, the surface charge (60, 61), the density of surface chemical groups (62, 63), and the elastic modulus (64), among others] can strongly influence in vivo pharmacokinetics (PK) (i.e., circulation times, uptake within specific cells or within specific organs or disease sites, clearance route, and toxicology) (65, 66). In particular, understanding which basic properties of nanomaterials can lead to nonspecific and potentially harmful biological interactions (67–69) in vivo is a very active field of study. In any case, this matrix of materials properties for optimization represents a sort of “opportunity hurricane” for the scientist or engineer. Efforts to elucidate how best to optimize NPs for specific tasks comprise much of the basic science of these NPs. Organizations such as the National Cancer Institute’s Nanotechnology Characterization Laboratory have played important roles in guiding such efforts (70). Although approved clinical applications are beginning to appear, the bulk of the science is still maturing through mouse model studies. In following paragraphs, I provide a very brief overview of this underlying science and highlight illustrative examples in Figs. 3 and 4.

Noble-metal NPs have been used for several years for the point-of-care detection of blood-based biomarkers from droplets of blood; gold NPs provide the colorimetric agents for analyte detection. The basic exploited physical property is the surface plasmon resonance (SPR), which is in the visible or near-visible part of the spectrum for noble-metal NPs. The SPR is a collective resonance that carries a very high oscillator strength, with a peak wavelength, line shape,

and intensity that strongly depend upon NP size, shape, and local dielectric environment. The basic optical properties of colloidal gold were described more than 100 y ago by Mie using Maxwell’s equations (71). Current models can capture the remarkable linear and nonlinear (72) optical properties achievable through modern synthetic methods in which surface chemistry, local chemical environment (73), and NP size and shape (74) are controlled. For in vitro biological applications, synthetic methods for surface-loading high-density coverages of DNA have enabled efficient cell delivery for various in-cell, live-cell assays (Fig. 3) (75). Other surface chemistries have enabled a myriad of electrochemical, chemical, and optical platforms for sensing and in vitro diagnostics (76–78). For in vivo applications, that synthetic control has been harnessed for minimizing toxicity [exhibited by some very small gold NPs (79)] and for targeting delivery of gold nanorods to specific tissues (80). Gold nanorods have shown promise in animal models for photothermal therapy applications. In such a therapy (81), the particles are delivered to the disease site, and laser light is used to penetrate into the tissue, excite the NPs, and transfer thermal energy to—and thus kill—the diseased tissue. This concept has had only limited success

in human trials (82) and so may need more time to mature.

Remarkable advances in nanomaterials synthesis have occurred for a broad variety of NPs, including magnetic NPs (83, 84). These advances have enabled applications of ferromagnetic and superparamagnetic NPs for both in vitro and in vivo (imaging) diagnostic assays (85, 86). For in vitro assays, biofunctionalized magnetic NPs have a history of use for magnet-assisted cell sorting. A recent variant of that application is the use of antibody-coated magnetic NPs for detecting (using a custom-built microchip-NMR tool) and sorting microvesicles released by certain solid tumors (87). Microvesicles are 10- to 100-fold smaller than the tumor cells from which they are released but can carry many of the biomarkers that identify the originating cell as a diseased cell (88). For certain tumors, such as glioblastomas, microvesicles are released into the blood, although circulating tumor cells are not.

Fig. 4 illustrates two in vivo live-animal imaging applications of highly fluorescent semiconductor QDs (Fig. 4*A*) (89) and near-IR fluorescing SWNTs (Fig. 4*B*) (90). In both cases, the nanomaterial exhibits a compelling optical property that enables the imaging application and also is highly engineered, through surface chemistry control, for useful PK and pharmacodynamics (PD) properties.

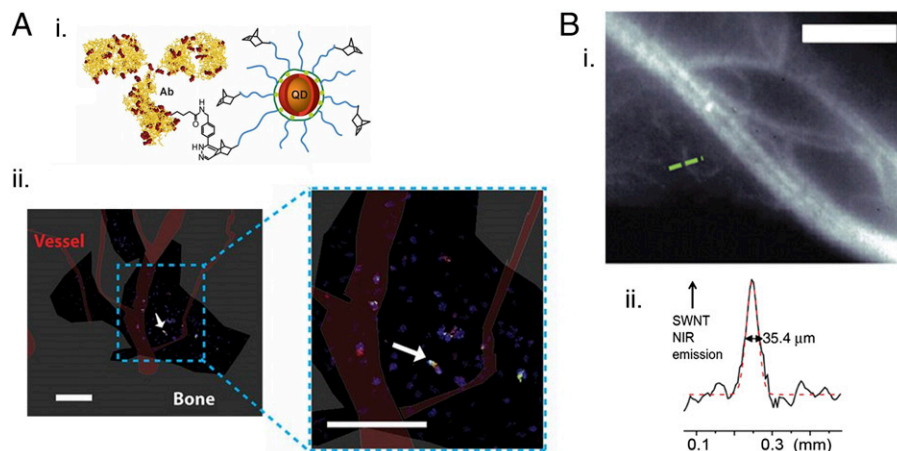


Fig. 4. Semiconductor QD and SWNT in vivo imaging probes. (A) Semiconductor QD imaging of stem cells in bone marrow. (i) Polyimidazole incorporating norbornene provides a bio-compatible surface functionalization for highly luminescent semiconductor core-shell QDs as well as a chemical handle for preparing QD–antibody conjugates, as shown (not to scale). Lysine (amine-presenting) residues on the antibody are highlighted in red. The QD–antibody particles exhibit a moderate negative surface charge, which is generally favorable for improved in vivo circulation. (ii) Use of the QD–antibody particles as in vivo imaging probes for single-cell imaging in the bone marrow of a live murine model, viewed through a calvarial window. The arrow points to single Sca-1⁺ c-Kit⁺ cell, which is a late-stage hematopoietic stem cell. The red and green cells represent Sca-1⁺ and c-Kit⁺ cells, respectively. Adapted from ref. 89. (B) Polymer-stabilized SWNTs used as in vivo near-infrared (NIR) fluorescent probes of vasculature. (i) Near-IR fluorescence of mouse vasculature. (ii) Fluorescence intensity taken along the dashed green line drawn on the image in *i* is plotted and reveals an imaging resolution of a few tens of micrometers. Adapted from ref. 90, with permission from Macmillan Publishers Ltd: *Nature Medicine*, copyright 2012.

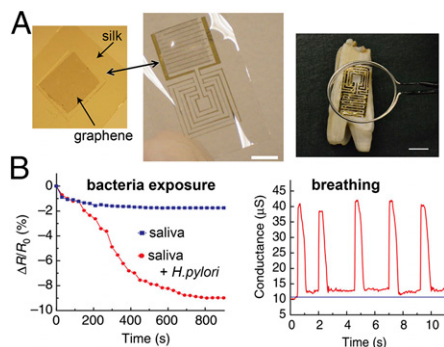


Fig. 7. Graphene nanotechnology integrated into flexible electronics yields a potentially wearable sensor platform. (A) Graphene is printed onto bioresorbable silk and then is electrically contacted with a small electrical circuit before transfer onto the surface of a tooth. The flexible circuit consists of interdigitated capacitive electrodes (to sense the graphene electrical conductivity) and a planar meander line inductor. The graphene is chemically modified with a bifunctional peptide to bind to the graphene and to exhibit affinity for specific bacteria. Exposure to bacteria modulates the electrical conductivity of the graphene, which is measured by the interdigitating electrodes and is transmitted wirelessly to an inductively coupled receiver. (B) Benchtop experiments show the ability of this sensor system to detect a specific biological agent (*Helicobacter pylori*) (Left) and a periodic physiological process (breathing) (Right). Adapted from ref. 124, with permission from Macmillan Publishers Ltd: *Nature Communications*, copyright 2012.

serving as carriers of the chemotherapeutic camptothecin are being explored currently in a number of human trials (92).

Nanomechanical and Nanoelectronic Devices and Wellness Monitoring

Goals nos. 2 and 6 of the 2000 NNI, which emphasized remote sensor systems and in vivo devices for early disease detection and diagnosis, represented interrelated grand challenges with somewhat similar roadmaps to success. Recent influences on these goals are the proliferation of smart phones and other smart devices as well as the emerging emphasis on wellness (105). A relevant emerging wellness trend is that an individual is provided with regular measurements of his/her health status. The individual can make lifestyle, exercise, and diet adjustments informed by those measurements so as to maximize health benefit. The technology challenge is to provide accurate, informative, and readily interpretable diagnostic measurements at low cost. The emerging science and technology of wearable health monitoring devices illustrates a dominant approach toward meeting this challenge. Nanotech does not play a role in the early-stage examples of such wearable devices, although it is clear that nanotech will play important roles in the near future. Current smart devices

monitor a panel of basic body functions, such as heart rate, body temperature, and exercise but, with few exceptions (106), do not monitor specific biomarker analytes. This current functionality is enabled by a menu of microelectromechanical machines (MEMs) such as microphones, accelerometers, and gyroscopes. The uptake of MEMs in consumer products was young in 2000, but of the three waves of proliferation initially envisioned for MEMs—automotive, consumer electronics, and home integration—the first two are now considered complete (107). A fourth wave, relevant to this discussion, is likely personal integration.

Recent advances (108–115) in integrating electronics onto low-elastic modulus polymer substrates and other moldable materials that are conformal, biocompatible, and, in some instances, even self-powered (116) are opening up a large menu of wearable devices based on mechanical, electronic, or electrochemical signal transduction (117, 118). A large fraction of these devices contain some nanotechnology, with a scientific basis that can be traced back to early demonstrations of nanotube (119) or nanowire (120, 121) sensors and the integration of nanowires and nanotubes onto plastic substrates (122, 123). The net result is that biomarker-rich liquids, such as sweat or saliva (124), can be used for continuous multiplex monitoring of health status-relevant analytes. An example of a nanotech-enabled tooth “decal” sensor is shown in Fig. 7. This device straddles the line between an implant and a wearable device. Although the presented data are from a benchtop experiment, they clearly illustrate the potential for real-time monitoring of both bodily functions (breathing) and analytes (bacteria in saliva).

Concluding Remarks: Nanomedicine and Emerging Challenges

In this Perspective, I have provided a partial view of the state of the art of nanomedicine. Unlike applications in fields such as energy or non-health-related biotech, biomedicine

has a unique and justifiably conservative set of rules for adopting new technologies for human use. Witness, for example, the remarkably rapid rise of CRISPR genome-editing technologies (and associated biotech companies) over the past 2 y. Witness also, however, the healthy scientific debate (125) and public (126) controversy that emerged when gene editing was carried out on non-viable human embryos (127). This response was warranted, but it means that gene-editing technologies have a long road to travel from the benchtop to the bedside. Nanomedicine faced similar challenges 15–20 y ago. It was a brand-new field, and the ability to tune physical or PK properties by tuning NP size and shape was a sparkling new addition to the synthetic toolkit. However, that addition was not well understood, and current good manufacturing practice (cGMP)-type manufacturing, which is a critical first step toward human health applications, was virtually unknown in nanotech. In addition, there were challenges unique to the field. The pool of young scientific talent that could work across the disciplines of chemistry, physics, materials science, engineering, and biomedicine didn’t exist, and most research institutions didn’t have the infrastructure to support that talent. All these challenges have been fully or partially addressed over the past 15 y. The number of nanotechnologies that currently are undergoing some level of human testing and the several areas in medicine in which nanotech is providing unique solutions are important measures of that success. Although the impact of this progress on the human health condition is still marginal, it will be felt increasingly over the next decade. Thus, as we look back at the distance traveled, it is indeed startling to find how far we have come.

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